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The influence of buspirone, and its metabolite 1-PP, on the activity of paroxetine in the mouse light/dark paradigm and four plates test

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Abstract

Although numerous animal procedures have been employed in the study of antidepressants (ADs) in anxiety, the results following acute administration remain highly variable. The present study investigated the effect of the SSRI paroxetine (4, 8, and 16 mg/kg, IP) in two tests of anxiety in mice: the light/dark test paradigm, and the four plates test (FPT). In both tests, it was found that paroxetine resulted in an anxiolytic-like effect at doses that did not modify motor performance (at the doses of 4 and 8 mg/kg in the light/dark test and at the doses of 4, 8, and 16 mg/kg in the four plates test). In the light/dark paradigm, both doses of buspirone significantly potentiated paroxetine, while in the four plates only one dose of buspirone (a $5HT_{1A}$ partial agonist) (0.06 mg/kg) increased the anxiolytic-like effect of paroxetine. Prior administration of 1-PP was without effect in the light/dark paradigm but antagonized the effect of paroxetine (at the dose of 0.06 and 0.5 mg/kg) in the FPT. The results suggested that a balance between pre- and postsynaptic 5-HT_{1A} receptor was implicated in the anxiolytic-like effect of paroxetine. Buspirone seemed to emphasize the role of paroxetine in 5-HT_{1A} receptor modulation and exerted a biphasic influence in the two tests.

Keywords: Paroxetine; Buspirone; 1-PP; Anxiety; Animal model; Acute administration; 5-HT_{1A} receptor; Mice

1. Introduction

The efficacy of antidepressants (ADs) as antianxiety treatment has been established for 10 years [2,16] and their effects on the central serotonin neuronal system are thought to mediate their efficacy [16,22,27,34,43]. Nevertheless, animal studies have been disappointing. The effect of ADs in different models of anxiety are highly variable, ranging from anxiogenic to inactive or anxiolytic-like activity [9,18,19,33,38,43,44]. Many studies reported that acute administration resulted in anxiogenic-like effects, while only chronic administration led to anxiolytic-like effects.

Buspirone has been shown to possess high affinity for central 5-HT1A receptors [29], and may be classified as a partial agonist at this receptor [28,35]. The underlying mechanism of action of buspirone is unclear. This azapirone, together with other members of its class (e.g., gepirone and ipsapirone), has been reported as being effective in several animal models of depression and anxiety [11,20,26,31,32,37,40] as well as attenuating the activity of ADs [15]. The major metabolite of buspirone, 1-(2-pyrimidinyl)-piperazine (1-PP or 1-PmP) is rapidly and abundantly formed in humans and rodents and tends to accumulate in brain [8], and has been shown to act as an α_2-adrenoreceptor antagonist in vitro and in vivo [7,42], but has no affinity for α_1-adrenoreceptors and dopamine receptors [42] and does not bind to 5-HT1A receptors [7]. α_2 antagonists have been shown to attenuate the antiimmobility effects of ADs in the forced swimming test (FST) [10].

A previous study [41] using the FST was performed to further investigate the mechanisms involved in the potential AD-enhancing effects of buspirone. Using several SSRIs including paroxetine, the results suggested that low-dose buspirone enhanced the activity of subactive doses of SSRIs in this test probably via an action at 5-HT_{1A} receptors, while a high dose of buspirone attenuated the AD-like effects of active doses of these drugs, possibly via the generation of the active metabolite 1-PP.

There is increasing evidence that SSRIs may be of potential therapeutic benefit for the treatment of anxiety,

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and several clinical studies have suggested that the 5-HT_{1A} receptor partial agonist buspirone may also be of use for treatment of anxiety. It seemed of interest to investigate such manipulation of the 5-HT neuronal system (i.e., association of paroxetine with a 5-HT_{1A} agonist) in animal models of anxiety and to compare the results with those found in the previous study performed [41]. In the purpose of clinical benefits of such association in clinical studies, buspirone was chosen, as it is the only 5-HT_{1A} agonist clinically available, even if it was not the most specific for these subtypes of receptors and it has an affinity for the D_2 receptor; however, it has been suggested that the D_2 receptor is not involved in the anxiolytic-like activity of buspirone [48]. Furthermore, previous studies have shown that the strongly specific 5-HT_{1A} receptor agonist 8-OH-DPAT does not possess anxiolytic-like activity in all behavioral models of anxiety in mice, including the light/dark paradigm [47].

Two tests of anxiety were chosen — the four plates test and the light/dark box test — which are based on different aversive factors. Many paradigms are aimed to study the anticonflict effect of anxiolytic drugs. Thus, in the four plates test, punishment is usually created by a mild electric shock when animals give a behavioral response, as, for example, an exploratory behavioral response [1,4,5,25]. The light/dark paradigm, however, is based on the natural aversion of rodents for brightly lit large spaces [13,14,23].

The present study was performed

(i) to further investigate the effect of acute administration of paroxetine in animal models of anxiety;
(ii) to examine the influence of the serotonin system, notably the 5-HT1A receptor, in the activity of paroxetine by the administration of buspirone prior to paroxetine;
(iii) to sensitize anxiety models to the effect of ADS;
(iv) to study the influence of buspirone's major metabolite, 1-PP.

2. Materials and methods

2.1. Animals

Male Swiss mice (4 weeks old) were purchased from R. Janvier (Le Genest, France). Their average body weight on the day of the study was 22 ± 2 g. These animals were housed in groups of 20, at constant temperature (20°C), with standard light cycle (lights on between 0700 and 1900 h), and had free access to food and water.

2.2. Drugs

Paroxetine HCl (SmithKline Beecham, France), buspirone HCl (Bristol-Myers, France), and 1-PP HCl (Aldrich, France). All drugs were ultrasonically dispersed in distilled water. All drugs or vehicle were administered IP in a volume of 0.5 ml/20 g of body weight. Control animals received vehicle only. Paroxetine was administered 30 min before testing. Pretreatment with buspirone or 1-PP was administered 45 min before testing. Mice were used only once.

The two doses of buspirone and 1-PP were chosen for use in interaction studies according to a previous study [41] in which buspirone or 1-PP were found to have no significant effects in the FST or locomotor activity test. A low dose of 0.06 mg/kg and a higher dose of 0.5 mg/kg were, therefore, chosen for each compound.

Four sessions of each test were performed, corresponding to the pretreatment of paroxetine with two doses of buspirone (0.06 and 0.5 mg/kg), respectively called Experiments 1 and 2, and with two doses of 1-PP (0.06 and 0.5 mg/kg), respectively called Experiments 3 and 4 (these two experiments were conducted at the same time).

2.3. Psychopharmacological tests

2.3.1. The "four plates" test

This apparatus consisted of a cage floored by four metal plates, connected to a device that generated electric shocks (0.6 mA, 0.5 s). Following a 15-s latency period, the animal was subjected to an electric shock after crossing from one plate to another. The number of crossings was recorded during a 1-min test period [1].

2.4. Light/dark exploration test in mice

2.4.1. Apparatus

The apparatus consisted of a fully automated box monitored by computer. It was constructed by OSYS, Orga system (Changé, France). The light/dark apparatus consisted of four Perspex test boxes, an RS 232C/ RS 422 interface together with software management of the experiments. An open-topped rectangular box ($46 \times 27 \times 30$ cm high), was divided into a small (18×27 cm) area and a large (27×27 cm) area with an opening door (7.5×7.5 cm) located in the center of the partition at floor level. The small compartment was painted black and illuminated under a dim red light (60 W, 4 lx), whereas the large compartment was painted white and brightly illuminated with a 60-W (400 lx) light source. The compartments were equipped with infrared beam sensors (four in the white area, three in the black one), enabling the detection of locomotion in each zone, time spent in each zone, latency of the first crossing from one compartment to the other and shuttle crossings between both compartments. The data from these four parameters were directly collected by computer.

2.4.2. Procedure

The test was performed in a quiet, darkened room. The mice were kept in this room at least 1 h before the

test. After injection (saline or treatment), mice were placed in their home cage. To reduce any neophobic response to the test situation, the light/dark compartments were previously soiled by mice other than those used during the test [23]. Mice were always tested in a soiled apparatus, and there was no cleaning between trials. Naive mice were placed individually in the middle of the light area facing away from the opening. A 5-min test was given, during which the four parameters were recorded [13,23].

2.4.3. Analysis of data

The mean number of responses for each group and for each test was calculated, and the final results were expressed as a percentage of the value observed in control animals or as a mean (with standard error of the mean in parentheses). For the analysis of movements in both compartments, data collected were expressed as movement by unity of time (movements/time spent in the area) to avoid false interpretation of results [23].

Normality of distribution was first examined using the nonparametric Kolmogorof–Smirnov test. Data was then subjected to an analysis of variance (ANOVA) according to the homogeneity of variances. Data were first transformed in rank if the homogeneity of variances did not permit a direct ANOVA analysis.

2.4.4. Post hoc tests

Interaction studies including paroxetine and buspirone were analyzed using the "a posteriori" Sidak test for multiple comparisons.

All analyses were conducted using the SPSS program for IBM-compatible computers.

The ethical rules of the French Ministry of Agriculture for experiments with laboratory animals (No. 87.848) were followed at all times.

3. Results

3.1. The four plates test

3.1.1. Effect of acute administration of paroxetine in the four plates test (Figs. 1 and 2)

The administration of paroxetine resulted in a dramatic increase in the number of punished crossings during the test, $F(7,152) = 40.628$; $p \leq 0.001$ [172±39% for 4 mg/kg, 200± 23% for 8 mg/kg, and 197±41% for 16 mg/kg in comparison with saline controls, $p \leq 0.001$ for each dose (Experiment 1)]. This effect was the same in the other experiments.

3.1.2. Interaction of buspirone 0.06 and 0.5 mg/kg with paroxetine (4, 8, and 16 mg/kg) in the four plates test (Fig. 1)

Prior administration of buspirone (0.06 mg/kg) significantly enhanced the punished crossings of the high dose of paroxetine, $F(7,152) = 40.628$, $p \leq 0.001$ (260± 66% vs. 197± 41% for paroxetine 16 mg/kg, $p \leq 0.05$) (Fig. 1).

However, pretreatment with the high dose of buspirone (0.5 mg/kg) did not induce any behavioral changes when tested in combination with the three doses of paroxetine.

3.1.3. Interaction of 1-PP 0.06 and 0.5 mg/kg with paroxetine in the four plates test (Fig. 2)

Pretreatment with 1-PP (0.06 mg/kg) did not modify behavior in paroxetine-treated mice, $F(7,152)=67.788$, $p \leq 0.001$ (Fig. 2).

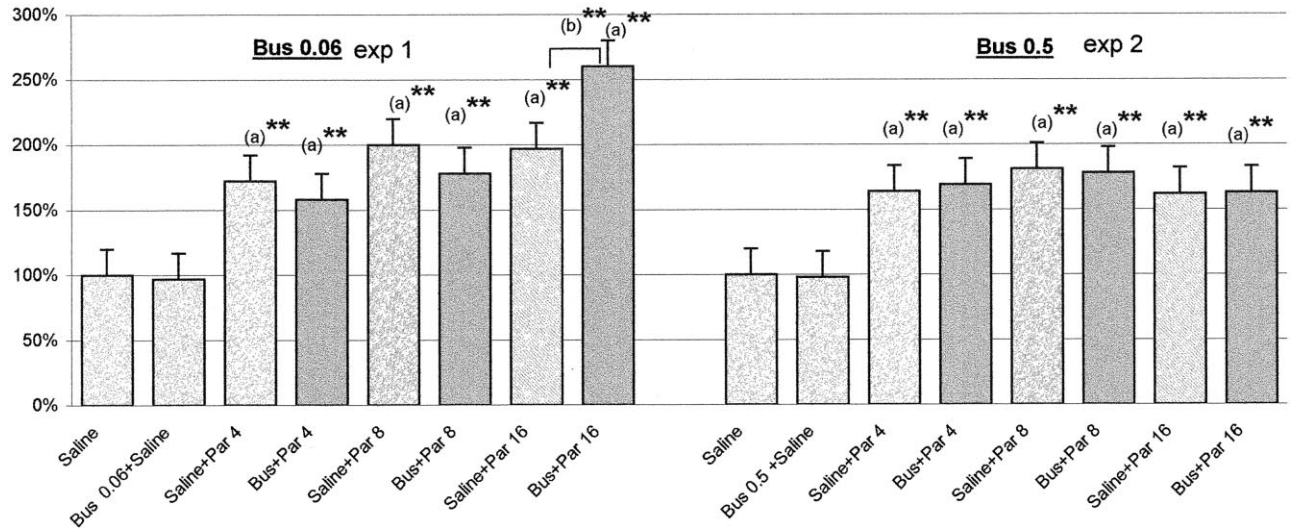

Fig. 1. Effect of prior administration of buspirone in paroxetine-treated mice in the four plates test. Results are expressed as the percentage of effect from control group ($n = 20$). Drugs were injected IP 30 min before the test for paroxetine and 45 min for buspirone. Statistical analyses were performed by application of an analysis of variance (ANOVA), followed by the a posteriori Sidak test for comparison with appropriate control group, $p \leq 0.05$* and $p \leq 0.01$** vs. (a) control group, or vs. (b) paroxetine alone.

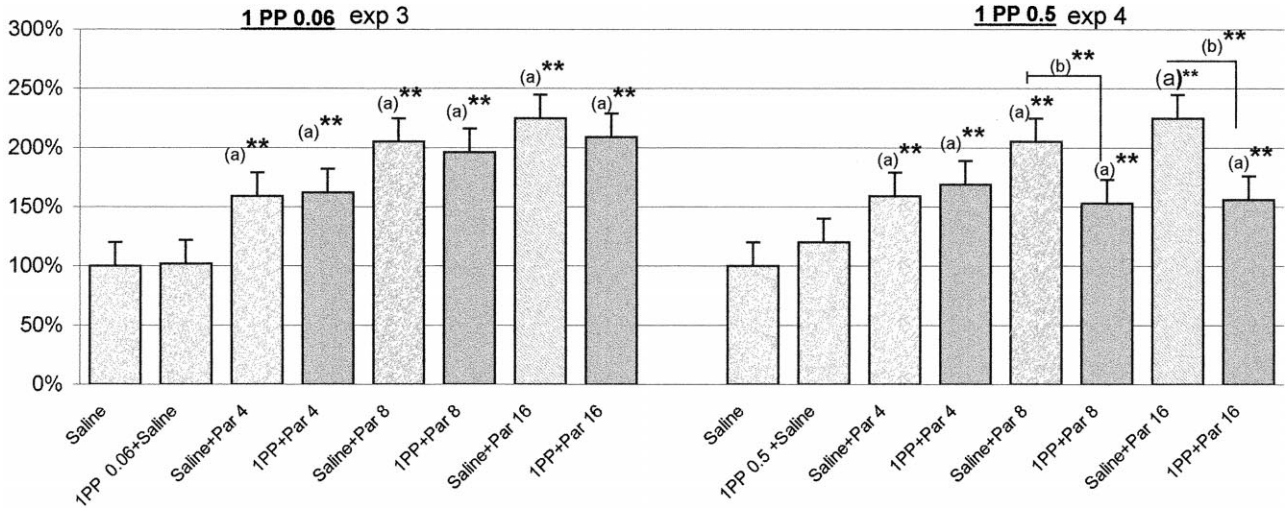

Fig. 2. Effect of prior administration of 1-PP in paroxetine-treated mice in the four plates test. Results are expressed as the percentage of effect from control group ($n = 20$). Drugs were injected IP 30 min before the test for paroxetine and 45 min for 1-PP. Statistical analyses were performed by application of ANOVA, followed by the a posteriori Sidak test for comparison with appropriate control group, $p \leq 0.05$* and $p \leq 0.01$** vs. (a) control group, or vs. (b) paroxetine alone.

The high dose of 1-PP dramatically reduced the increase in punished crossings induced by paroxetine (8 mg/kg), $F(7,152) = 44.012$, $p \leq 0.001$ (153 ± 31% vs. 205 ± 32%, $p \leq 0.001$) and 16 mg/kg (156 ± 25% vs. 225 ± 29%, $p \leq 0.001$).

3.2. The light/dark test

3.2.1. Effect of acute administration of paroxetine in the light/dark test (Tables 1 and 2)

Treatment with paroxetine weakly reduced time spent in the dark compartment for the doses of 4, 8, and 16 mg/kg in Experiment 2, $F(7,232) = 10.101$, $p \leq 0.001$ ($p \leq 0.001$for multiples comparisons) and for the dose of 4 mg/kg in Experiment 1, $F(7,152) = 5.683$, $p \leq 0.001$, the doses of 8 and 16 mg/kg were limit significant, $p = 0.142$ and $p = 0.06$, respectively. In Experiments 3 and 4, results did not reach statistical significance, $F(7,152) = 2.596$, $p \leq 0.015$, and, $F(7,152)=1.690$, $p \leq 0.028$, respectively. An increase in the number of transitions was seen in Experiment 1. For all doses tested an increase in movements in each compartment was observed in all experiments. The latency time to enter the dark compartment tended to be enhanced by paroxetine treatment for the doses of 16 mg/kg (Experiments 3 and 4) and 8 mg/kg (Experiment 2). The increase did not reach statistical significance in Experiment 1.

3.2.2. Interaction of buspirone 0.06 and 0.5 mg/kg with paroxetine (Table 1)

Buspirone, at the doses of 0.06 and 0.5 mg/kg did not induce "anxiolytic like" effects when administered alone (Table 1). Prior administration of buspirone (0.06 mg/kg) significantly potentiated the reduction in time spent in the dark compartment induced by the 8 mg/kg dose of paroxetine, $F(7,152)=5.683$, $p \leq 0.001$ (44.22% for interaction vs. 51.73% for control paroxetine, $p < 0.05$). The administration of buspirone (0.06 mg/kg) was without effect at either 4 or 16 mg/kg. For the 8 mg/kg of paroxetine, this effect was associated with a weak decrease in activity in the light compartment, $F(7,152) = 6.870$, $p \leq 0.001$. No effect was observed for the latency time parameter. The same tendency was observed in all parameters for the two other doses of paroxetine.

The higher dose of buspirone (0.5 mg/kg) displayed the same profile of activity as buspirone (0.06 mg/kg). Pretreatment with buspirone reduced time spent in the dark compartment for the dose of 4 and 8 mg/kg, $F(7,232) = 10.101$, $p \leq 0.001$ (45% vs. 54% for control paroxetine $p \leq 0.01$, and 46% vs. 49%, respectively). The number of transitions was weakly decreased for the dose of 4 mg/kg of paroxetine, $F(7,232) = 3.270$, $p \leq 0.02$ ($p = 0.08$ vs. controls). Activity in each compartment was also reduced when paroxetine was administered with buspirone (0.05 mg/kg). The latency time was also reduced, but the effect did not reach statistical significance.

3.2.3. Interaction of 1-PP 0.06 and 0.5 mg/kg with paroxetine (Table 2)

Pretreatment with 1-PP 0.06 mg/kg weakly reduced time spent in the dark compartment (Table 2). However, data did not reach statistical significance, $F(7,152) = 2.596$, $p \leq 0.015$ (43.2 vs. 47.87% for paroxetine 16 mg/kg), but was significantly different from saline controls ($p \leq 0.05$). Activity in both compartments was not influenced by prior administration of 1-PP (0.06 mg/kg) with the exception of pretreatment with 1-PP 0.06 mg/kg for the activity in the dark area ($p \leq 0.01$), the number of transitions between the

Table 1
Light/dark test parameters means (SEM)

Groups	Latency		Movements by unity time		
	L → D	Transitions	Dark	Light	% Time in D/300
Buspirone 0.06 mg/kg (Experiment 1)					
saline + saline	19.5	13.80	0.72	0.84	60.37%
	1.46	1.10	0.04	0.04	1.65
bus 0.06 + saline	16.75	15.95	0.93 (a) **	0.88	56.45%
	1.95	0.51	0.03	0.04	1.97
saline + par 4	24.95	21.30 (a) **	1.17 (a) **	1.07 (a) **	49.20% (a) **
	1.94	0.84	0.05	0.04	1.63
bus 0.06 + par 4	20.65	18.20	1.12 (a) **	0.97	46.70% (a) **
	1.56	1.15	0.06	0.04	1.70
saline + par 8	21.4	21.60 (a) **	1.14 (a) **	1.20 (a) **	51.73%
	1.82	0.88	0.04	0.05	2.28
bus 0.06 + par 8	27	19.3	1.14 (a) **	0.98	44.22% (a) **
	4.20	2.10	0.06	0.05	2.37 (b) *
saline + par 16	24.35	18.95	1.18 (a) **	1.18 (a) **	52.28%
	2.2	1.33	0.07	0.09	3.43
bus 0.06 + par 16	28.10	15.35	1.01 (a) **	0.93	51.60%
	3.75	1.32	0.06	0.04 (b) *	2.79
$F(7,152)$	2.007; $p \leq 0.049$	5.182; $p \leq 0.001$	8.916; $p \leq 0.001$	6.870; $p \leq 0.001$	5.683; $p \leq 0.001$
Buspirone 0.5 mg/kg (Experiment 2)					
saline + saline	18.20	16.53	0.79	0.86	59%
	1.25	0.72	0.03	0.02	1.48
bus 0.5 + saline	22.10	17.37	1.04 (a) **	0.92	53.0%
	1.85	0.70	0.03	0.03	1.24
saline + par 4	22.53	18.60	1.06 (a) **	1.00	54%
	1.70	0.92	0.05	0.04	1.63
bus 0.5 + par 4	25.53	15.50	1.00 (a) **	0.86	45% (a) **
	1.77	0.71	0.03	0.04 (b) *	1.76 (b) **
saline + par 8	24.20	19.70	1.16 (a) **	1.09 (a) **	49% (a) **
	1.79	0.79	0.04	0.03	1.89
bus 0.5 + par 8	28.03 (a) **	18.10	1.07 (a) **	1.00	46% (a) **
	2.05	0.91	0.04	0.03	1.25
saline + par 16	24.60	19.57	1.23 (a) **	1.07 (a) **	45.0% (a) **
	1.90	0.83	0.04	0.03	1.71
bus 0.5 + par 16	29.27 (a) **	17.17	1.08 (a) **	1.00	46.68% (a) *
	1.87	0.85	0.05	0.03	1.51

Effects of prior administration of buspirone in paroxetine-treated mice on behavioral parameters in the light/dark test in mice: drugs were injected, IP, respectively 45 and 30 min before the light/dark test ($n = 20$). Statistical analyses were performed by application of ANOVA, followed by the a posteriori Sidak test for comparison with appropriate control group, $p \leq 0.05$* and $p \leq 0.01$** vs. (a) control group, or vs. (b) paroxetine alone (D = dark compartment; L = light compartment).

two compartments was also not modified. The latency time to enter the dark area was reduced, $F(7,152) = 4.936$, $p \leq 0.001$ (24.35 vs. 34.34 s).

Pretreatment with the high dose of 1-PP (0.5 mg/kg) did not induce any behavioral changes when tested in combination with the three doses of paroxetine.

4. Discussion

The present study examined the effects of acute paroxetine and paroxetine in association with buspirone or its major metabolite 1-PP. With this purpose, two different behavioral procedures were used, i.e., the four plates test and light/dark test. In both tests, it was found that acute administration of paroxetine resulted in an anxiolytic-like effect at doses that did not modify the animals' motor performance (see previous study, Ref. [41]). However, results of the light/dark test were disappointing because of the great variability of data. After appropriate statistical analysis, many data were nearly significant. The effect was more controversial and highly dependent on the control mice values (in Experiments 1 and 2, see Table 1, the effect was more significant than in Experiments 3 and 4, Table 2). The emotional state of mice might influence the effect of paroxetine treatment in the exploratory test. Discrepancy between the effects of SSRIs could be due to the fact that animal models of anxiety represent qualitatively different type of fear or "anxiety" [22].

Our findings were in contrast to many other animal studies, and clinical findings that suggested that SSRIs, when given acutely, did not reduce experimental anxiety

Table 2
Light/dark test parameters means (SEM)

	Latency		Movements/unity time		
Groups	L → D	Transitions	Dark	Light	% Time in D/300
1 PP 0.06 mg/kg (Experiment 3)					
saline+saline	20.5	16.90	0.90	0.82	53.20%
	1.74	0.86	0.04	0.03	2.01
1 PP 0.06+saline	17.15	15.75	0.92	0.84	54.50%
	2.39	0.83	0.04	0.04	1.69
saline + par 4	25.5	17.35	1.06	1.04 (a) **	50.33%
	2.42	1.10	0.06	0.05	2.58
1 PP 0.06+par 4	25.65	17.90	1.15 (a) *	0.95	48.63%
	2.20	0.89	0.06	0.03	2.26
saline+par 8	24.15	19.25	1.16 (a) *	1.07 (a) **	48.72%
	1.91	1.10	0.05	0.06	2.22
1 PP 0.06+par 8	19.60	18.05	1.12	0.99	46.90%
	1.33	1.16	0.07	0.06	2.80
saline+par 16	34.35 (a) **	17.60	1.07	1.02 (a) *	47.87%
	3.16	1.16	0.05	0.05	1.79
1 PP 0.06+par 16	24.35	18.60	1.30 (a) **	1.02 (a) *	43.20% (a) *
	1.82	0.86	0.07	0.04	2.14
$F(7,152)$	4.936; $p \leq 0.001$	1.123; $p \leq 0.351$	5.351; $p \leq 0.001$	4.785; $p \leq 0.001$	2.596; $p \leq 0.015$
1 PP 0.5 mg/kg (Experiment 4)					
saline+saline	20.50	16.90	0.90	0.82	53.20%
	7.76	3.82	0.17	0.15	2.01
1 PP 0.5+saline	21.80	15.70	1.02	0.94	54.37%
	10.39	2.81	0.14	0.28	1.63
saline+par 4	25.50	17.35	1.06	1.04 (a) *	50.33%
	10.84	4.92	0.28	0.22	2.58
1 PP 0.5+par 4	25.20	16.70	1.06	1.00	51.77%
	11.50	4.29	0.32	0.19	2.61
saline+par 8	24.15	19.25	1.16 (a) *	1.07	48.72%
	8.53	4.92	0.20	0.29	2.22
1 PP 0.5+par 8	29.00	17.15	1.11	0.90 (a) **	46.20%
	13.51	3.28	0.23	0.15	1.56
saline+par 16	34.05 (a) *	17.60	1.07	1.02	47.87%
	14.15	5.21	0.24	0.23	1.79
1 PP 0.5+par 16	24.40	17.75	1.21 (a) **	1.08 (a) **	49.40%
	11.19	5.04	0.24	0.16	2.27
$F(7,152)$	2.910; $p \leq 0.07$	1.078; $p \leq 0.380$	3.181; $p \leq 0.004$	4.033; $p \leq 0.001$	1.690; $p \leq 0.001$

Effects of prior administration of 1-PP in paroxetine treated mice on behavioral parameters in the light/dark test in mice: drugs were injected, IP, respectively 45 and 30 min before the light/dark test ($n = 20$). Statistical analyses were performed by application of ANOVA, followed by the a posteriori Sidak test for comparison with appropriate control group, $p \leq 0.05$* and $p \leq 0.01$** vs. (a) control group, or vs. (b) paroxetine alone (D = dark compartment; L = light compartment).

as the symptoms of GAD or panic disorders [3,33,38]. Indeed, studies have frequently reported that acute administration of SSRIs elicits anxiogenic-like responses. Sanchez and Meier [45], studying the behavioral profile of five SSRIs including paroxetine, found that citalopram produced a mixed anxiogenic/anxiolytic-like response in the light dark test in rats, and paroxetine induced an anxiogenic-like response at low doses. This could conceivably be the result of stimulating different receptor subtypes. After acute administration, citalopram also depressed the firing activity of dorsal serotoninergic neurones, parallel to the blockade of 5-HT reuptake [12,45]. These effects added to the confusing hypothesis of activity of SSRIs in animal models of anxiety.

It had been found [43] that citalopram facilitated exploratory activity in the white compartment. In this study, it was suggested that the biphasic dose–response curve (facilitation vs. decrease in exploratory behavior) of citalopram was a function of the 5-HT net effect resulting from the stimulation of receptor subtypes mediating either anxiolytic-like or anxiogenic-like responses.

In the four plates test, paroxetine induced strong anxiolytic-like effects. The substantial difference between the two tests in terms of the type of stressor (exploratory vs. foot shock) consequently induced the involvement of different cerebral regional structures [17,30], and hence, differences in receptor stimulation (e.g., pre- vs. postsynaptic receptors). A recent study [34], using burying behavior in which

the aversive stimulus was directly presented to the animal (electric shocks) vs. the light/dark paradigm, demonstrated that the effect of buspirone on 5-HT_{1A} pre- or postreceptors stimulation highly depended on the nature of the aversive stimulus and on the expression of the behavior. One main idea developed from our results, as well as from the studies of Sanchez and Meier [45] and Lopez-Rubalcava [34] was that the major difference in SSRI activity in animal anxiety models was linked to the nature of the stress. Two different types of responses seemed to correspond to punished procedure via electric shock (four plates test, burying behavior, and shock-induced ultrasonic vocalization) vs. natural aversion (like in the light/dark procedure). Furthermore, the duality of effect of 5-HT_{1A} receptor activation was dependent on the neuroanatomical localization of the receptors, and the 5-HT pathways that are activated might depend on the behavioral test condition [17]. In a recent study [24], ADs with different mechanisms of action, including tricyclics, selective serotonin reuptake inhibitors (SSRIs), a monoamine oxidase inhibitor (MAOI), and atypicals, were studied in the FPT to evaluate their anxiolytic-like effects following acute administration. The number of punished crossings was dramatically increased by the SSRIs citalopram, fluvoxamine, and paroxetine but not fluoxetine. The mixed 5-HT/NE reuptake inhibitors, milnacipran and venlafaxine, also demonstrated strong antipunishment effects. The specific NE reuptake inhibitors, desipramine and maprotiline, and the atypical AD trazodone, enhanced freezing behavior suggesting anxiogenic-like behavior. It was concluded that, in the FPT, a model based on spontaneous response, where animals are exposed to an aversive environment from which they can only escape by being motionless, this kind of behavior might be related to anticipatory anxiety. In this situation, ADs acting preferentially on 5-HT transmission possessed clear anxiolytic like effects.

Pretreatment with buspirone could help to understand if the 5-HT_{1A} receptor subtype is implicated in the effect of SSRIs and if activation of this receptor could sensitize the tests. In the light/dark paradigm, both doses of buspirone (0.06 and 0.5 mg/kg) significantly potentiated paroxetine 8 mg/kg (buspirone 0.06 mg/kg) and 4 and 8 mg/kg (buspirone 0.5 mg/kg), respectively. However, the effect with coadministration of paroxetine 8 mg/kg and buspirone 0.05 mg/kg was weak. In the four plates test, only buspirone 0.06 mg/kg potentiated the 16 mg/kg dose of paroxetine. Taken together these results suggest that 5-HT_{1A} receptors are implicated in the processes by which paroxetine induces anxiolytic-like effects. However, the mechanisms remain unclear [46]. Using the FST, it was found in a previous study that low doses of buspirone (0.06 mg/kg) enhanced the effect of subactive doses of SSRIs including paroxetine (4 mg/kg) [40]. Conversely, a high dose of buspirone (0.5 mg/kg) was found to antagonize active doses of SSRIs, including paroxetine 16 mg/kg. Similarly, DaRocha et al. [15] demonstrated using the FST that coadministration of buspirone (0.5 mg/kg) and SSRIs resulted in a decrease in mobility time (buspirone 0.5 mg/kg with paroxetine 8 mg/kg). It was proposed that stimulation of 5-HT_{1A} autoreceptors, by buspirone, reduced the ability of the SSRI fluoxetine to enhance 5-HT transmission at the postsynaptic level. Therefore, increasing the inhibitory feedback system would result in a global reduction in serotonin synthesis and release that leads to a reduction in anxiety. Furthermore, Lopez-Rubalcava [34] clearly demonstrated that in tests dependent on direct punishment, such as the defensive burying test, the activity of buspirone was mediated via postsynaptic receptors. This could, therefore, also occur in the analogous four plates test. On the other hand, in a passive-avoidance paradigm, like the light/dark test, the buspirone anxiolytic-like effect might be due to a presynaptic autoreceptor mechanism.

The findings of the present study suggest that the major metabolite 1-PP, an α_2 adrenoreceptor antagonist [39], which is known to accumulate in the brain at higher concentrations than its parent compounds [6–8,10], also plays a role in the effects of the coadministration of buspirone and paroxetine. Indeed, when 1-PP (0.5 mg/kg) was administrated prior to paroxetine in the four plates test, it strongly attenuated the anxiolytic-like effect seen in this test. Nevertheless, no effect was observed in the light/dark paradigm with a 1-PP pretreatment, suggesting that the metabolite did not participate in the paroxetine response in this test. On the other hand, in the FST, 1-PP (0.5 mg/kg) reduced the immobility time of 16 mg/kg paroxetine-treated mice [41]. Antagonism of paroxetine response was mediated by 1-PP probably via the α_2-adrenoreceptor, as in the four plates test. Another hypothesis is possible, as it has recently been reported that 1-PP depressed the excitatory amino acid mediated transmission via (probably) the activation of 5-HT_{1A} subtype receptors [36]. Further experiments are needed to clarify such mechanisms.

In conclusion, the results presented in this study have demonstrated that prior administration of buspirone highly influences the effect of the SSRI paroxetine. Buspirone also exerted a biphasic influence in two tests, the four plates test (this study), and the FST [41]. For low doses (0.06 mg/kg) it enhanced the effect of paroxetine per se. For the higher dose (0.5 mg/kg) it had no effect in the four plates test. Buspirone seemed to emphasize the role of paroxetine in 5-HT1A receptor modulation. Furthermore, the four plates test seemed more sensitive to the high dose of paroxetine with a 16-mg/kg active dose, while a lower active dose was seen in the light/dark test. The 8-mg/kg dose of paroxetine was perhaps a turning point for the balance between pre- and postsynaptic activity. Finally, Griebel [21] hypothesized that anxiety models are not equivalent, and more than one 5-HT mechanism may be involved, depending on the stress of the aversive events being controlled or uncontrolled. Our

results suggested that paroxetine might act on both anxiety types, depending on the dose.

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